Biochimica et Biophysica Acta 1777 (2008) 929–934

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Looking for the minimum common denominator in haem–copper oxygen reductases: Towards a unified catalytic mechanism

Manuela M. Pereira *, Filipa L. Sousa, Andreia F. Veríssimo, Miguel Teixeira

Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República – EAN, 2780-157 Oeiras, Portugal

ARTICLE INFO

Article history:
Received 1 February 2008
Received in revised form 15 April 2008
Accepted 16 May 2008
Available online 26 May 2008

Keywords:
Haem–copper oxygen reductase
Cytochrome oxidase
Quinol oxidase
Electron–proton coupling

ABSTRACT

Haem–copper oxygen reductases are transmembrane protein complexes that reduce dioxygen to water and pump protons across the mitochondrial or periplasmatic membrane, contributing to the transmembrane difference of electrochemical potential. Seven years ago we proposed a classification of these enzymes into three different families (A, B and C), based on the amino acid residues of their proton channels and amino acid sequence comparison, later supported by the so far identified characteristics of the catalytic centre of members from each family. The three families have in common the same general structural fold of the catalytic subunit, which contains the same or analogous prosthetic groups, and proton channels. These observations raise the hypothesis that the mechanisms for dioxygen reduction, proton pumping and the coupling of the two processes may be the same for all these enzymes. Under this hypothesis, they should be performed and controlled by the same or equivalent elements/events, and the identification of retained elements in all families will reveal their importance and may prompt the definition of the enzyme operating mode. Thus, we believe that the search for a minimum common denominator has a crucial importance, and in this article we highlight what is already established for the haem–copper oxygen reductases and emphasize the main questions still unanswered in a comprehensive basis.

1. Common characteristics of haem–copper oxygen reductases

Enzymes belonging to the haem–copper superfamily are membrane-bound protein complexes that catalyse the complete reduction of dioxygen to water. Since the electrons and protons needed for this reaction are delivered from opposite sides of the membrane (positive and negative sides, respectively), these oxygen reductases promote charge separation that contributes to the establishment of a transmembrane difference of electrochemical potential. Moreover, these enzymes are able to utilize part of the free energy associated with the favourable electron transfer from the primary electron donors (cytochromes *c* or other metalloproteins, with E=~250 mV, or quinols, with E>−90 mV) to dioxygen (E=~800 mV), to promote proton translocation across the membrane, further contributing in this way to the difference of electrochemical potential. Thus, besides being O_2 reductases, haem–copper enzymes are also proton pumps. The superfamily is defined by the presence, in subunit I (the catalytic subunit), of a six-coordinated low-spin haem and a bimetallic centre (which gives the name to this family) composed by a high-spin haem and a copper ion, Cu_B. Dioxygen reduction takes place at this binuclear site [1–4] (Fig. 1). Due to their structural similarities, NO reductases were recently considered as members of this superfamily [5]. However, these enzymes catalyse a different reaction, the reduction of NO to N_2O, and have in their catalytic site an iron ion instead of the copper ion, Cu_B; thus we do not consider the haem–iron NO reductases as members of the haem–copper superfamily, in spite of the several analogies between O_2 and NO reductases [6,7]. We also prefer to call the haem–copper enzymes as oxygen reductases and not cytochrome oxidases, as most frequently used in the literature, because all of them are indeed O_2 reductases, but only some are cytochrome oxidases. Apart from cytochromes, high potential iron–sulphur proteins (HiPIPs) [8], copper proteins [9,10] or quinols may be electron donors for this type of enzymes.

Besides the common catalytic subunit I, the members of the haem–copper superfamily have extra subunits composing their minimal functional unit. In the case of enzymes receiving electrons from a periplasmatic electron transport protein, with the exception of the cbb_3 oxygen reductases, subunit II contains a prosthetic group composed by two copper ions forming an average valence binuclear site, named Cu_A. Some of these enzymes contain a C-terminal extension, harbouring at least one haem C [2]. In the case of quinol oxidases no prosthetic group is present in this subunit. Extra subunits, namely subunit III, seem to play also important roles, yet to be fully clarified [11,12]. The cbb_3 oxygen reductases have three extra subunits besides the catalytic one, two of them containing additional redox centres, a dihaemic and a monohaemic cytochromes (Table 1).

Up to date five X-ray crystallographic structures of haem–copper reductases have been determined [13–17]. The general fold of the catalytic subunit is remarkably similar. The same fold is also expected for the other members of the superfamily based on sequence alignments

* Corresponding author. Tel.: +351 214469321; fax: +351 214428314.
E-mail address: mpereira@itqb.unl.pt (M.M. Pereira).

0005-2728/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2008.05.441

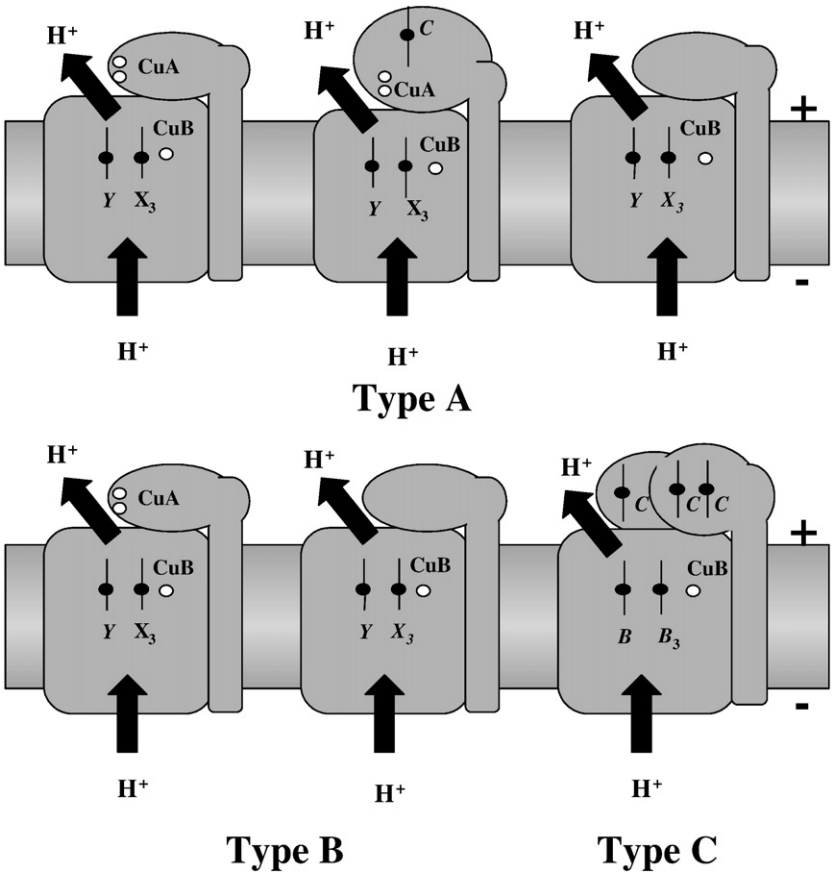

Fig. 1. Schematic representation of the main types of haem–copper oxygen reductases belonging to the A, B and C families. Proton pathways are generally represented by thick arrows; X and Y stand for haems A, B or O or their derivatives, depending on the enzyme.

and homology modelling [8,18,19]. Since protons are chemical and pump substrates for all these enzymes, intramolecular proton conducting pathways have to be present. Based on sequence alignments, site directed mutations and later on the crystallographic structures, two proton channels (D- and K-channels) were identified for mitochondrial and mitochondrial-like enzymes. However, a detailed analysis of the available amino acid sequences and structural information revealed important differences among the haem–copper enzymes, regarding particularly their proton channels.

2. Classification of haem–copper oxygen reductases[1]

Seven years ago we proposed a classification of haem–copper oxygen reductases based on sequence alignments and conserved crucial amino acid residues, which make part of the proton pathways [2]. Remarkably out of approximately 600 amino acid residues, only the six hystidyls, which are ligands of the prosthetic groups (haems and Cu_B) are strictly conserved in the catalytic subunit. The members of the haem–copper superfamily were grouped into three families, named A, B and C, one of which comprising two subfamilies designated A1 and A2 [2] (Fig. 1, Table 1).

2.1. Type A family[2]

The mitochondrial enzyme and its close relatives are members of this family, which is further divided in two subfamilies named A1 and A2. The A1 subfamily is constituted by oxygen reductases having the D- and K-channels first observed for the mitochondrial-like enzymes. Besides AspI-124 (D) close to the negative side of the membrane, after which the channel is called, the D-channel contains hydrophilic amino acid residues (AsnI-199, AsnI-113, AsnI-131, TyrI-35, SerI-134, SerI-193) ending at a glutamyl, GluI-278, considered to be a key residue for the operating mechanism of haem–copper enzymes. The residues LysI-354 (K), ThrI-351, SerI-291 and TyrI-280 are part of the K-channel (e.g. [3,13,14,20,21]). This last tyrosyl is covalently bound to one of the histidyl ligands of Cu_B (HisI-276), and has been proposed to play an important role in the catalytic cycle, namely in the heterolytic splitting of the O_2 molecule [22–24]. In the members of the subfamily A2 all the residues considered essential for the D- and K-channels (above mentioned) are present, with the exception of the helix VI glutamyl (GluI-278). A homology based three dimensional (3D) model of *Rhodothermus* (*R.*) *marinus* caa_3 oxygen reductase suggested that a tyrosine residue, in a position equivalent to PheI-274, i.e., one helix turn below the glutamate residue in helix VI, and whose hydroxyl group was predicted to occupy the spatial place of the carboxyl group of the glutamyl, may participate in proton transfer [8,25,26]. Furthermore, these enzymes also contain a conserved consecutive serine residue, which may be also relevant for proton transfer; this so-called YS motif is the fingerprint of the members of the type A2 subfamily [2].

2.2. Type B family

In the members of the type B family the residues composing the D- and K-channels in type A enzymes are not conserved. However, a K-channel homologue with a threonine, a serine and a tyrosine residues replacing LysI-354, ThrI-351 and SerI-291, respectively, could be functional. The tyrosyl covalently bound to the histidyl coordinating

[1] Throughout the text, and unless otherwise stated, the amino acid numbering of *Paracoccus denitrificans* aa_3 oxygen reductase, is used.

[2] The amino acid residues defined for each channel are the ones considered to be important for the catalytic and/or pumping mechanisms in A type enzymes.

Table 1
Characteristics of members of haem–copper oxygen reductases families and subfamilies

Oxygen reductases family or subfamily	A		B	C
	A1	A2		
Examples	*P. denitrificans* aa_3	*T. thermophilus* caa_3	*T. thermophilus* ba_3	*V. cholerae* cbb_3
	E. coli bo_3	*R. marinus* caa_3	*A. ambivalens* aa_3	*R. sphaeroides* cbb_3
	Bovine aa_3	*Synechocystis* sp aa_3	*R. marinus* ba_3	*B. japonicum* cbb_3
Minimal functional unit	*Subunit I*	Low-spin haem (A, B and A derivatives)		Low-spin haem (B)
		High-spin haem (A, O and derivatives)		High-spin haem (B)
		Cu_B		Cu_B
	Subunit II	Cu_A or Cu_A and low-spin haem C		FixO/CcoO[a]
		No prosthetic groups (quinol oxidases)		1 Low-spin haem C
	Subunit III	No prosthetic groups		FixP/CcoP[a]
				2 Low-spin haems C
Proton channels	D-channel	D-channel		
	(E)	(YS)		
	K-channel	K-channel	"K-channel"	"K-channel"
Pumping stoichiometry in reconstituted enzymes	0.75–1 H^+/e	0.8–1 H^+/e	0.5–0.75 H^+/e	0.2–0.4 H^+/e
Intermediates identified by Resonance Raman	P (Fe^{IV}=O)	nd	nd	nd
	F (Fe^{IV}=O)[b]			
Tyr-His crosslink	+		+	+

nd, not determined; "K-channel", alternative K-channel.
[a] The FixO/CcoO and FixP/CcoP subunits found in C type enzymes are not related with subunits II and III from the enzymes belonging to the A and B families.
[b] These two intermediates may differ on the protonation state of a nearby protonable centre.

Cu_B is also present in these enzymes. The ba_3 oxygen reductase from *Thermus* (*T.*) *thermophilus* is the only member of this family whose crystallographic structure has been determined [15]. It was suggested that apart from the alternative K-channel, there are two other possible proton channels in this enzyme; however, their functionality has still to be shown. Furthermore, inspection of the amino acid sequence alignment of the enzymes from the type B family shows that none of the amino acid residues (or equivalent ones), constituent of those putative channels is common among the members of this family [2]. For the aa_3 oxygen reductase from *Acidianus* (*A.*) *ambivalens* a pseudo D-channel was suggested on the basis of a structural model [27], but again this putative channel was not observed in other members of this family.

A recent analysis of a large number of the now available amino acid sequences of haem–copper enzymes led to the proposal of more families, from D to H [5]. The members of these new proposed families, whose sequences were available at the time of our first proposal, were all included in the type B family [2]. Indeed, the amino acid sequences of the newly proposed family members have in common the same alternative K-channel present in the enzymes of the type B family and seem to have the same characteristics (independently of their electron donors), such as the relative order of the reduction potential of the haems [28,29] and the properties of the binuclear centre (reviewed in [30]). We would like to stress that more divisions and subdivisions should be taken with a greater precaution, because in many cases only one example was so far predicted and, furthermore, a very detailed classification may become an overclassification and may lead to the loss of the general concept of grouping the enzymes based on relevant functional and structural properties. Throughout the article we will maintain our classification of the haem–copper oxygen reductases in three different families (A, B and C).

2.3. Type C family

This family only comprises cbb_3 oxygen reductases. These reductases apparently have only part of the alternative K-channel observed for the members of the B type family, with a seryl and a tyrosyl in the place of the ThrI-351 and SerI-291. There is not a tyrosine residue in the same sequence position as TyrI-280, the tyrosyl covalently bound to a copper histidyl ligand, but homology models suggested that a tyrosine residue in another helix, helix VII, could have an equivalent function, being also covalently bound to that copper histidyl ligand [18,19]. In fact the hystidyl–tyrosyl crosslink was recently identified by mass spectrometry [31,32]. None of the canonical residues of the D-channel is present in these enzymes [2]. Also based on a homology 3D model, and on site directed mutants it was suggested that only one proton channel is present in type C oxygen reductases [33].

A particularly important conclusion from the comparison of the 3D structures and structural models of several haem–copper oxygen reductases, as well as of their primary sequences, is that many of these enzymes do not have any protonable residues in between their surface facing the inner membrane side and the catalytic centre, where protons are needed for the catalytic reaction [34]. This observation clearly indicates the importance of water molecules in proton conduction. It should be emphasized that in crystal structures are only observable, at best, water molecules well oriented, and for most cases there is structural data only for the oxidized enzyme, which may not reflect the distribution of water molecules inside the protein during catalysis. Several calculations have shown that the water content in O_2 reductases is much higher than that detected by X-ray crystallography, as it happens in many other enzymatic systems. Equally important is the fact that the water molecules have a high degree of mobility, both inside the protein, including hydrophobic regions, and exchanging with the environment [35–37]. Thus, without detailed structural, mutagenesis (including the structural determination of the mutants), functional and theoretical analysis, it is quite premature to define how many proton channels are indeed functional in each enzyme. Also, water channels may be formed only transiently, which makes a precise definition of proton conducting pathways quite difficult.

3. Proton pumping

All members of the haem–copper superfamily pump protons, although with different stoichiometries. The stoichiometry is not 1 H^+/e for all the members of the superfamily, and even the same enzyme has not a fixed stoichiometry in all conditions, as generally considered in the literature when discussing coupling mechanisms. For several A1 type oxygen reductases, including the mitochondrial and quinol bo_3 oxidase from *Escherichia* (*E.*) *coli*, the stoichiometries obtained are in the range of 0.75 to 1 H^+/e [38–41] and differ according to the pH of the medium [40–43]. Two reports of proton pumping by A2 type enzymes indicated that they pump protons with a stoichiometry of 0.8–1 H^+/e [44,45]. For the B type oxygen reductases, proton pumping has been

observed for the *T. thermophilus* ba_3 [46] and *Geobacillus stearothermophilus* $b(o)a_3$ cytochrome oxidases [47], and for the *A. ambivalens* aa_3 quinol oxidase [48]. While for the first two enzymes a stoichiometry of ca 0.5 H^+/e was measured, for the *A. ambivalens* enzyme a ratio of ~0.75 H^+/e was obtained. Proton pumping by the C type (cbb_3) oxygen reductases has also been demonstrated, using either whole cells from *P. denitrificans* and *Rhodobacter sphaeroides* [49,50] with a H^+/e stoichiometry between 0.6 and 1, or with the purified *Bradyrhizobium japonicum* cbb_3 oxygen reductase reconstituted in artificial liposomes [51], in which case the maximum pumping stoichiometry was 0.4 H^+/e.

It should be mentioned that the values of the stoichiometries available in the literature were obtained under different experimental conditions including pH values, enzyme "status" and ionic strength. Also relevant for such different stoichiometries are the lipids used during the experiments, which in most cases are distinct from those of the respective organisms. With such a large set of variables, the values published should be taken as indicative and not definitive (Table 1).

4. Catalytic intermediates

The investigation of the nature of the catalytic intermediates has been addressed for members of the A1 subfamily. Six intermediate states have been proposed, R (reduced binuclear centre), A (adduct, O_2 bound to the reduced binuclear centre), P (although called Peroxy for historical reasons, it is indeed a ferryl, Fe^{IV}=O), F (Ferryl, Fe^{IV}=O), O (oxidized binuclear centre) and E (one electron at the binuclear centre), but their chemical composition is still not fully established (e.g., [3,4,52,53]), namely in terms of protonation states and nature of possible participating radicals. For the members of the other A subfamily and families the catalytic intermediates have still to be investigated (Table 1). We have also observed previously that the properties of the binuclear centre in what respects to its interaction with ligands, such as CO and reactivity with NO seems to differ between the members of the different families (reviewed in [30]). However, these studies have only been performed with few enzyme representatives, in several cases with only one, and do not allow to make extrapolations or generalizations.

5. Thermodynamic properties

For a redox-driven proton pump, in which the main events are electron transfer processes and their coupling to proton movements, most probably associated to protonation/deprotonation events, a detailed determination of the redox behaviour of each redox centre and the pH influence on this behaviour are important parameters that must sustain any model for the enzymatic mechanism. Although studied since a long time, the data available is still quite limited, and analysed using methodologies not generally described in sufficient detail, or even inappropriate, such as the use of several Nernst equations to describe one electron transitions. What is well accepted and common to all cases studied so far is that the haems interact in a way that the reduction of one of the haems decreases the electronic affinity of the other (e.g., [54]). However, there are strong discrepancies regarding the relative magnitudes of the midpoint reduction potentials: although it is generally assumed that both the low-spin and the high-spin haems have close potentials, there are already a considerable number of experimental data that clearly show that this is not the case; even the relative order of the potentials may be different from enzyme to enzyme [28,55,56]. Moreover, the pH dependence of the reduction potentials has been interpreted on the basis of equations such as $E = E_{\text{acid}} + \frac{RT}{F} \ln\left(\frac{K_{\text{Red}} + [H^+]}{K_{\text{Ox}} + [H^+]}\right)$. This type of equation is only valid in a system with a single redox centre in the presence of a single protonable group. In the case of haem–copper reductases more than one redox centre is present, and more than one protonable group within the protein is expected to interact with them [57–59]. The observed pH dependence of the reduction potentials results from the effect of multiple partial protonation/deprotonation events, eventually also associated with conformational changes that affect the local electrostatics. How relevant these effects are on the overall mechanism remains to be established, but certainly the assumption of *iso*-potential centres is not confirmed by the data; therefore, these facts have to be taken into consideration in any theoretical analysis of the pumping mechanisms.

6. Does the search for a minimum common denominator make sense?

All members of haem–copper oxygen reductases perform the same functions: reduce dioxygen to water and pump protons. All are predicted to have the same general structural fold of the catalytic subunit, the same type of prosthetic groups in it, and proton channels. This means that all should have the same structural/functional relation and so it is plausible to hypothesize that the chemical reaction, proton translocation and their coupling mechanisms may be the same. Thus, the search for a minimum common denominator will reveal the elements strictly necessary for those mechanisms and, on the other hand, the identification of such elements will prompt the establishment of the operating mode of haem–copper oxygen reductases.

Looking for a minimum common denominator is a simple rationale, but not a simplistic or an easy approach. The rationale considers that if a property, structural and/or functional, is retained in all families it has to be relevant for the functioning of the haem–copper enzymes. This view, may also be considered reductionist, but in fact it is global, as it will describe the general rules of oxygen reducing and proton pumping mechanisms in all members of the haem–copper superfamily, i.e., in K. Popper's sense, the more general is a scientific theory or model, the strongest it will be, and the easiest will be to prove or disprove it. Of course, such a general model does not exclude the importance of specific elements such as the glutamyl present at the end of the D-channel of type A1 enzymes [36,57,60–63]. However, we think that these family-type specific elements are upgrades or a fine-tuning of a confined group of enzymes and cannot be invocated (simply because they, or equivalent elements are not present) to be determinant for the general operating mechanism of haem–copper oxygen reductases. The models presently under discussion [36,57,60–69] are restricted, being formulated considering, for example, the presence of the "key" glutamate residue of the D-channel, a fixed pumping stoichiometry of 1 H^+/e and isoexergonicity of the catalytic steps, considering that the events hypothesized for the first loaded electron are the same for all subsequent electron inputs. Also, the K-channel is less or not even considered. The role of the glutamyl may stand, of course, for the enzymes in which it is present. But even in those cases, it remains to be shown firmly whether this residue plays mainly an electrostatic role, influencing nearby residues and water molecules, or if it really undergoes protonation/deprotonation events. The consideration of a fixed proton/electron stoichiometry is also not valid since there are already several examples in the literature in which different stoichiometries are observed depending on several factors, such as the pH [40–43]. Isoexergonicity of the several catalytic steps may not be observable, since the several catalytic intermediates are chemically and thus electrostatically different, which will affect the electron and proton affinities of the relevant intervenients in each catalytic intermediate.

Although many studies on haem–copper oxygen reductases have been performed, more data for the different members of the superfamily, even for the members of A1 type family, are still needed. This makes it too premature even to suggest a general operating model for these enzymes. Many questions are still to be answered such as: the entering and exiting pathways for protons, including the

role of specific amino acid residues and of water molecules; the variability of proton/electron stoichiometries; the chemical nature(s) of the catalytic intermediates for the different enzyme families; the role of the membrane potential in controlling the function of the haem–copper enzymes and the importance of the local membrane structure on the enzyme behaviour.

A general model may only be proposed after an extensive structural and functional characterization of several members of the different families of the haem–copper enzymes, which will reveal possible common elements. This is only achievable through a thorough spectroscopic, structural, thermodynamic and kinetic characterization of a good sampling of enzymes, comprising haem–copper oxygen reductases from phylogenetic distant organisms.

Thus, the search for a minimal common denominator may be determinant to establish the basic operating mode of haem–copper oxygen reductases.

Acknowledgments

Filipa L. Sousa and Andreia F. Veríssimo are recipients of grants from Fundação para a Ciência e a Tecnologia, SFRH XXI/BD/27972/2006 and SFRH XXI/BD/14388/2003, respectively. This work was supported by Fundação para a Ciência e a Tecnologia (PTDC/QUI/66559/2006 to MMP; POCI/BIA-PRO/58608/2004 to MT).

References

[1] S. Ferguson-Miller, G.T. Babcock, Heme/copper terminal oxidases, Chem. Rev. 7 (1996) 2889–2907.
[2] M.M. Pereira, M. Santana, M. Teixeira, A novel scenario for the evolution of haem–copper oxygen reductases, Biochim. Biophys. Acta 1505 (2001) 185–208.
[3] J.P. Hosler, S. Ferguson-Miller, D.A. Mills, Energy transduction: proton transfer through the respiratory complexes, Annu. Rev. Biochem. 75 (2006) 165–187.
[4] M. Wikstrom, Cytochrome c oxidase: 25 years of the elusive proton pump, Biochim. Biophys. Acta 1655 (2004) 241–247.
[5] J. Hemp, R.B. Gennis, Diversity of the heme-copper superfamily in archaea: insights from genomics and structural modeling, Results Probl. Cell Differ. 45 (2008) 1–31.
[6] W.G. Zumft, Nitric oxide reductases of prokaryotes with emphasis on the respiratory, heme-copper oxidase type, J. Inorg. Biochem. 99 (2005) 194–215.
[7] I.M. Wasser, S. de Vries, P. Moenne-Loccoz, I. Schroder, K.D. Karlin, Nitric oxide in biological denitrification: Fe/Cu metalloenzyme and metal complex NO(x) redox chemistry, Chem. Rev. 102 (2002) 1201–1234.
[8] M.M. Pereira, M. Santana, C.M. Soares, J. Mendes, J.N. Carita, A.S. Fernandes, M. Saraste, M.A. Carrondo, M. Teixeira, The caa3 terminal oxidase of the thermohalophilic bacterium *Rhodothermus marinus*: a HiPIP:oxygen oxidoreductase lacking the key glutamate of the D-channel, Biochim. Biophys. Acta 1413 (1999) 1–13.
[9] L. Komorowski, W. Verheyen, G. Schafer, The archaeal respiratory supercomplex SoxM from *S. acidocaldarius* combines features of quinol and cytochrome c oxidases, Biol. Chem. 383 (2002) 1791–1799.
[10] J.A. Navarro, R.V. Duran, M.A. De la Rosa, M. Hervas, Respiratory cytochrome c oxidase can be efficiently reduced by the photosynthetic redox proteins cytochrome c_6 and plastocyanin in cyanobacteria, FEBS Lett. 579 (2005) 3565–3568.
[11] E.O. Ogunjimi, C.N. Pokalsky, L.A. Shroyer, L.J. Prochaska, Evidence for a conformational change in subunit III of bovine heart mitochondrial cytochrome *c* oxidase 1, J. Bioenerg. Biomembr. 32 (2000) 617–626.
[12] J.P. Hosler, The influence of subunit III of cytochrome c oxidase on the D pathway, the proton exit pathway and mechanism-based inactivation in subunit I, Biochim. Biophys. Acta 1655 (2004) 332–339.
[13] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A, Science 272 (1996) 1136–1144.
[14] S. Iwata, C. Ostermeier, B. Ludwig, H. Michel, Structure at 2.8 A resolution of cytochrome c oxidase from *Paracoccus denitrificans*, Nature 376 (1995) 660–669.
[15] T. Soulimane, G. Buse, G.B. Bourenkov, H.D. Bartunik, R. Huber, M.E. Than, Structure and mechanism of the aberrant ba_3-cytochrome *c* oxidase from *Thermus thermophilus*, EMBO J. 19 (2000) 1766–1776.
[16] J. Abramson, S. Riistama, G. Larsson, A. Jasaitis, M. Svensson-Ek, L. Laakkonen, A. Puuustinen, S. Iwata, M. Wikstrom, The structure of the ubiquinol oxidase from *Escherichia coli* and its ubiquinone binding site, Nat. Struct. Biol. 7 (2000) 910–917.
[17] M. Svensson-Ek, J. Abramson, G. Larsson, S. Tornroth, P. Brzezinski, S. Iwata, The X-ray crystal structures of wild-type and EQ(I-286) mutant cytochrome c oxidases from *Rhodobacter sphaeroides*, J. Mol. Biol. 321 (2002) 329–339.
[18] J. Hemp, C. Christian, B. Barquera, R.B. Gennis, T.J. Martinez, Helix switching of a key active-site residue in the cytochrome cbb_3 oxidases, Biochemistry 44 (2005) 10766–10775.
[19] V. Sharma, A. Puustinen, M. Wikstrom, L. Laakkonen, Sequence analysis of the cbb_3 oxidases and an atomic model for the *Rhodobacter sphaeroides* enzyme, Biochemistry 45 (2006) 5754–5765.
[20] R.B. Gennis, Multiple proton-conducting pathways in cytochrome oxidase and a proposed role for the active-site tyrosine, Biochim. Biophys. Acta 1365 (1998) 241–248.
[21] H. Michel, J. Behr, A. Harrenga, A. Kannt, Cytochrome *c* oxidase: structure and spectroscopy, Annu. Rev. Biophys. Biomol. Struct. 27 (1998) 329–356.
[22] M.R. Blomberg, P.E. Siegbahn, G.T. Babcock, M. Wikstrom, O–O bond splitting mechanism in cytochrome oxidase, J. Inorg. Biochem. 80 (2000) 261–269.
[23] G. Buse, T. Soulimane, M. Dewor, H.E. Meyer, M. Bluggel, Evidence for a copper-coordinated histidine-tyrosine cross-link in the active site of cytochrome oxidase, Protein Sci. 8 (1999) 985–990.
[24] D.A. Proshlyakov, M.A. Pressler, C. DeMaso, J.F. Leykam, D.L. DeWitt, G.L. Babcock, The missing link in cytochrome oxidase: Y_{244} is redox-active in O_2 activation and reduction, Science 2000 (2000) 1588–1591.
[25] M. Santana, M.M. Pereira, N.P. Elias, C.M. Soares, M. Teixeira, Gene cluster of *Rhodothermus marinus* high-potential iron–sulfur protein: oxygen oxidoreductase, a caa_3-type oxidase belonging to the superfamily of heme–copper oxidases, J. Bacteriol. 183 (2001) 687–699.
[26] M.M. Pereira, F.L. Sousa, M. Teixeira, R.M. Nyquist, J. Heberle, A tyrosine residue deprotonates during oxygen reduction by the caa_3 reductase from *Rhodothermus marinus*, FEBS Lett. 580 (2006) 1350–1354.
[27] B.L. Victor, A.M. Baptista, C.M. Soares, Theoretical identification of proton channels in the quinol oxidase aa_3 from *Acidianus ambivalens*, Biophys. J. 87 (2004) 4316–4325.
[28] F.L. Sousa, A.F. Verissimo, A.M. Baptista, T. Soulimane, M. Teixeira, M. Pereira, Redox properties of *Thermus thermophilus* ba_3: different electron–proton coupling in oxygen reductases? Biophys. J. 94 (2008) 1–8.
[29] S. Todorovic, M.M. Pereira, T.M. Bandeiras, M. Teixeira, P. Hildebrandt, D.H. Murgida, Midpoint potentials of hemes *a* and a_3 in the quinol oxidase from *Acidianus ambivalens* are inverted, J. Am. Chem. Soc. 127 (2005) 13561–13566.
[30] M.M. Pereira, M. Teixeira, Proton pathways, ligand binding and dynamics of the catalytic site in haem–copper oxygen reductases: a comparison between the three families, Biochim. Biophys. Acta 1655 (2004) 340–346.
[31] J. Hemp, D.E. Robinson, K.B. Ganesan, T.J. Martinez, N.L. Kelleher, R.B. Gennis, Evolutionary migration of a post-translationally modified active-site residue in the proton-pumping heme–copper oxygen reductases, Biochemistry 45 (2006) 15405–15410.
[32] V. Rauhamaki, M. Baumann, R. Soliymani, A. Puustinen, M. Wikstrom, Identification of a histidine-tyrosine cross-link in the active site of the cbb_3-type cytochrome *c* oxidase from *Rhodobacter sphaeroides*, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 16135–16140.
[33] J. Hemp, H. Han, J.H. Roh, S. Kaplan, T.J. Martinez, R.B. Gennis, Comparative genomics and site-directed mutagenesis support the existence of only one input channel for protons in the C-family (cbb_3 oxidase) of heme–copper oxygen reductases, Biochemistry 46 (2007) 9963–9972.
[34] M.M. Pereira, C.M. Gomes, M. Teixeira, Plasticity of proton pathways in haem–copper oxygen reductases, FEBS Lett. 522 (2002) 14–18.
[35] E. Olkhova, M.C. Hutter, M.A. Lill, V. Helms, H. Michel, Dynamic water networks in cytochrome *c* oxidase from *Paracoccus denitrificans* investigated by molecular dynamics simulations, Biophys. J. 86 (2004) 1873–1889.
[36] M. Wikstrom, M.I. Verkhovsky, G. Hummer, Water-gated mechanism of proton translocation by cytochrome *c* oxidase, Biochim. Biophys. Acta 1604 (2003) 61–65.
[37] J. Xu, G.A. Voth, Redox-coupled proton pumping in cytochrome *c* oxidase: further insights from computer simulation, Biochim. Biophys. Acta 1777 (2008) 196–201.
[38] A.S. Pawate, J. Morgan, A. Namslauer, D. Mills, P. Brzezinski, S. Ferguson-Miller, R.B. Gennis, A mutation in subunit I of cytochrome oxidase from *Rhodobacter sphaeroides* results in an increase in steady-state activity but completely eliminates proton pumping, Biochemistry 41 (2002) 13417–13423.
[39] O.M. Richter, K.L. Durr, A. Kannt, B. Ludwig, F.M. Scandurra, A. Giuffre, P. Sarti, P. Hellwig, Probing the access of protons to the K pathway in the *Paracoccus denitrificans* cytochrome *c* oxidase, FEBS J. 272 (2005) 404–412.
[40] M.L. Verkhovskaya, A. Garcia-Horsman, A. Puustinen, J.L. Rigaud, J.E. Morgan, M.I. Verkhovsky, M. Wikstrom, Glutamic acid 286 in subunit I of cytochrome bo_3 is involved in proton translocation, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10128–10131.
[41] G. Capitanio, P.L. Martino, N. Capitanio, E. De Nitto, S. Papa, pH dependence of proton translocation in the oxidative and reductive phases of the catalytic cycle of cytochrome *c* oxidase. The role of H_2O produced at the oxygen-reduction site, Biochemistry 45 (2006) 1930–1937.
[42] G. Antonini, F. Malatesta, P. Sarti, M. Brunori, Proton pumping by cytochrome oxidase as studied by time-resolved stopped-flow spectrophotometry, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 5949–5953.
[43] N. Capitanio, G. Capitanio, E. De Nitto, S. Papa, Vectorial nature of redox Bohr effects in bovine heart cytochrome *c* oxidase, FEBS Lett. 414 (1997) 414–418.
[44] M.M. Pereira, M.L. Verkhovskaya, M. Teixeira, M.I. Verkhovsky, The caa_3 terminal oxidase of *Rhodothermus marinus* lacking the key glutamate of the D-channel is a proton pump, Biochemistry 39 (2000) 6336–6340.
[45] K. Hon-nami, T. Oshima, Purification and characterization of cytochrome *c* oxidase from *Thermus thermophilus* HB8, Biochemistry 23 (1984) 454–460.
[46] A. Kannt, T. Soulimane, G. Buse, A. Becker, E. Bamberg, H. Michel, Electrical current generation and proton pumping catalyzed by the ba_3-type cytochrome *c* oxidase from *Thermus thermophilus*, FEBS Lett. 434 (1998) 17–22.
[47] N. Sone, S. Koyanagi, J. Sakamoto, Energy-yielding properties of SoxB-type cytochrome bo_3 terminal oxidase: analyses involving *Bacillus stearothermophilus* K1041 and its mutant strains, J. Biochem. 127 (2000) 551–557.
[48] C.M. Gomes, C. Backgren, M. Teixeira, A. Puustinen, M.L. Verkhovskaya, M. Wikstrom, M.I. Verkhovsky, Heme–copper oxidases with modified D- and K-pathways are yet efficient proton pumps, FEBS Lett. 497 (2001) 159–164.

[49] J.W. de Gier, M. Lubben, W.N. Reijnders, C.A. Tipker, D.J. Slotboom, R.J. van Spanning, A.H. Stouthamer, J. van der Oost, The terminal oxidases of *Paracoccus denitrificans*, Mol. Microbiol. 13 (1994) 183–196.
[50] M. Toledo-Cuevas, B. Barquera, R.B. Gennis, M. Wikstrom, J.A. Garcia-Horsman, The cbb_3-type cytochrome *c* oxidase from *Rhodobacter sphaeroides*, a proton-pumping heme–copper oxidase, Biochim. Biophys. Acta 1365 (1998) 421–434.
[51] E. Arslan, A. Kannt, L. Thony-Meyer, H. Hennecke, The symbiotically essential cbb_3-type oxidase of *Bradyrhizobium japonicum* is a proton pump, FEBS Lett. 470 (2000) 7–10.
[52] F.G. Wiertz, O.M. Richter, B. Ludwig, S. de Vries, Kinetic resolution of a tryptophan-radical intermediate in the reaction cycle of *Paracoccus denitrificans* cytochrome *c* oxidase, J Biol. Chem. 282 (2007) 31580–31591.
[53] M.H. Olsson, P.E. Siegbahn, M.R. Blomberg, A. Warshel, Exploring pathways and barriers for coupled ET/PT in cytochrome *c* oxidase: a general framework for examining energetics and mechanistic alternatives, Biochim. Biophys. Acta 1767 (2007) 244–260.
[54] M. Wikström, K. Krab, M. Saraste, Cytochrome Oxidase — a Synthesis, Academic Press, New York, 1981.
[55] A.F. Verissimo, F.L. Sousa, A.M. Baptista, M. Teixeira, M.M. Pereira, Thermodynamic redox behavior of the heme centers of cbb_3 heme–copper oxygen reductase from *Bradyrhizobium japonicum*, Biochemistry 46 (2007) 13245–13253.
[56] E.A. Gorbikova, K. Vuorilehto, M. Wikstrom, M.I. Verkhovsky, Redox titration of all electron carriers of cytochrome *c* oxidase by Fourier transform infrared spectroscopy, Biochemistry 45 (2006) 5641–5649.
[57] D.M. Popovic, A.A. Stuchebrukhov, Electrostatic study of the proton pumping mechanism in bovine heart cytochrome *c* oxidase, J. Am. Chem. Soc. 126 (2004) 1858–1871.
[58] A. Kannt, C.R. Lancaster, H. Michel, The coupling of electron transfer and proton translocation: electrostatic calculations on *Paracoccus denitrificans* cytochrome *c* oxidase, Biophys. J. 74 (1998) 708–721.
[59] C.M. Soares, A.M. Baptista, M.M. Pereira, M. Teixeira, Investigation of protonatable residues in *Rhodothermus marinus* caa_3 haem–copper oxygen reductase: comparison with *Paracoccus denitrificans* aa_3 haem–copper oxygen reductase, J. Biol. Inorg. Chem. 9 (2004) 124–134.
[60] P.E. Siegbahn, M.R. Blomberg, Energy diagrams and mechanism for proton pumping in cytochrome *c* oxidase, Biochim. Biophys. Acta 1767 (2007) 1143–1156.
[61] J. Quenneville, D.M. Popovic, A.A. Stuchebrukhov, Combined DFT and electrostatics study of the proton pumping mechanism in cytochrome *c* oxidase, Biochim. Biophys. Acta 1757 (2006) 1035–1046.
[62] E. Fadda, C.H. Yu, R. Pomes, Electrostatic control of proton pumping in cytochrome *c* oxidase, Biochim. Biophys. Acta 1777 (2008) 277–284.
[63] G. Branden, A.S. Pawate, R.B. Gennis, P. Brzezinski, Controlled uncoupling and recoupling of proton pumping in cytochrome *c* oxidase, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 317–322.
[64] H. Michel, The mechanism of proton pumping by cytochrome *c* oxidase, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12819–12824.
[65] A.V. Xavier, Thermodynamic and choreographic constraints for energy transduction by cytochrome *c* oxidase, Biochim. Biophys. Acta 1658 (2004) 23–30.
[66] T. Tsukihara, K. Shimokata, Y. Katayama, H. Shimada, K. Muramoto, H. Aoyama, M. Mochizuki, K. Shinzawa-Itoh, E. Yamashita, M. Yao, Y. Ishimura, S. Yoshikawa, The low-spin heme of cytochrome *c* oxidase as the driving element of the proton-pumping process, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15304–15309.
[67] S. Papa, G. Capitanio, P. Luca Martino, Concerted involvement of cooperative proton–electron linkage and water production in the proton pump of cytochrome *c* oxidase, Biochim. Biophys. Acta 1757 (2006) 1133–1143.
[68] Y.C. Kim, M. Wikstrom, G. Hummer, Kinetic models of redox-coupled proton pumping, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2169–2174.
[69] K. Kamiya, M. Boero, M. Tateno, K. Shiraishi, A. Oshiyama, Possible mechanism of proton transfer through peptide groups in the H-pathway of the bovine cytochrome *c* oxidase, J Am. Chem. Soc. 129 (2007) 9663–9673.